DATA EVALUATION RECORD

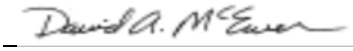
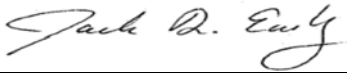
GLYPHOSATE

Study Type: OCSPP 890.1600, *In vivo* Uterotrophic Assay

EPA Contract No. EP10H001452
Task Assignment No. 2-34-2012 (MRID 48617003)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
CSS-Dynamac Corporation
1910 Sedwick Road,
Building 100, Suite B
Durham, NC 27713

Primary Reviewer: Kelly Luck, M.S.	Signature: Date:	 4/11/2012
Secondary Reviewer: David A. McEwen, B.S.	Signature: Date:	 4/13/2012
Program Manager: Jack D. Early, M.S.	Signature: Date:	 4/18/2012
Quality Assurance: Jack D. Early, M.S.	Signature: Date:	 4/18/2012

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CSS-Dynamac Corporation personnel**.**

The US EPA Endocrine Disruptor Screening Program (EDSP) Tier 1 screening battery is comprised of eleven screening assays intended to identify a chemical's likely endocrine bioactivity, i.e., its potential to interact with the estrogen, androgen, or thyroid (E, A, or T) pathways. The robustness of the Tier 1 battery is based on the strengths of each individual assay to identify potential endocrine bioactivity with complementary endpoints within the assay, where available, and redundancy across the battery. Thus, the results of each individual assay should not be considered in isolation but rather should be considered in the context of other assays in the battery as well as Other Scientifically Relevant Information (OSRI). In order to determine if a chemical has the potential to interact with the E, A or T pathways, a Weight of Evidence (WoE) evaluation of Tier 1 assay results, in combination with the findings in the OSRI, should be undertaken (refer to the WoE Document).

Primary Reviewer: Anwar Y. Dunbar, Ph.D. **Signature:** _____
Risk Assessment Branch 1, Health Effects Division (7509P) **Date:** _____
Secondary Reviewer: Jess Rowland **Signature:** _____
Health Effects Division (7509P) **Date:** _____

Template version 09/2011

DATA EVALUATION RECORD

STUDY TYPE: Uterotrophic Assay (Rat); OCSPP 890.1600; OECD 440

PC CODE: 417300 **DP BARCODE:** D398693

TXR#: 0053233 **CAS#:** 1071-83-6

TEST MATERIAL (PURITY): Glyphosate (85.1% a.i.)

SYNONYMS: N-(phosphonomethyl) glycine

CITATION: Stump, D. G. (2012). A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Report No.: WIL-843002, January 6, 2012. MRID 48617003. Unpublished.

SPONSOR: Joint Glyphosate Task Force, LLC, 8325 Old Deer Trail, Raleigh, NC 27615

TEST ORDER #: CON-417300-23

EXECUTIVE SUMMARY: In a Uterotrophic Assay (MRID 48617003) conducted to screen for potential estrogenic activity, glyphosate (85.1% a.i., Batch/lot# GLP-1103-21149-T) in 0.5% methylcellulose (w/v) was administered daily via oral gavage to groups of six ovariectomized female Sprague Dawley rats at dose levels of 0 (vehicle), 100, 300, or 1,000 (limit dose) mg/kg/day on post-natal days (PND) 66/67 to 68/69. The positive control group was treated with a daily dose of 17α-ethynyl estradiol (EE) at 3 µg/kg/day by oral gavage. Body weights were determined daily. All animals were terminated and necropsied on PND 69/70 approximately 24 hours after the final dose administration to determine wet and blotted uterine weights.

All animals survived until scheduled termination and no treatment-related clinical findings were observed in glyphosate dosed animals. Body weights, body weight gains, and uterine weights in the glyphosate groups were comparable to the vehicle control.

In the positive control (EE) group, mean body weights decreased on Days 3 and 4 (not significant, NS), leading to an overall body weight loss during the study of 5.6 g ($p<0.01$) compared to a gain of 11.3 g in the controls. Absolute wet and blotted uterus weights for the positive control (EE) group were increased ($p<0.01$) by 758% and 256%, respectively, as expected.

No statistically significant changes were seen in uterine weight in this assay. Glyphosate is negative in the uterotrophic assay.

The assay **satisfies** the EDSP Tier 1 Test Order requirements for a uterotrophic assay (OCSPP 890.1600).

COMPLIANCE: Signed and dated GLP Compliance, Data Confidentiality and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

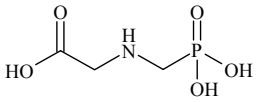
A. MATERIALS

1. Test Facility: WIL Research Laboratories, LLC

Location: Ashland, OH
Study Director: D. G. Stump
Other Personnel: E. S. Bodle (Assistant Director, Analytical Chemistry), S. A. Keets (Senior Operations Manager, Vivarium), C. A. Kopp (Manager, Gross Pathology and Developmental Toxicology Laboratory), T. M. Rafeld (Group Manager, Formulations Laboratory), C. S. Wally (Group Supervisor, Sample Processing Laboratory), R. A. Wally (Operations Manager, Reporting & Technical Support Services), M. E. Haubenstricker (Participating Scientist, Analyses of Dosing Formulations), L. Freshwater (Contributing Scientist, Statistical Analysis)
Study Period: June 14, 2011 - January 6, 2012

2. Test Substance: Glyphosate

Description: White powder
Source: Monsanto (St. Louis, MO)
Lot/Batch #: GLP-1103-21149-T (expiration date 3/9/2012)
Purity: 85.14% (95.93% dried)
Stability: Stable in vehicle for up to 15 days at room temperature
CAS #: 1071-83-6
Structure:



3. Reference Estrogen: 17α-ethynyl estradiol (EE)

Supplier: Sigma Aldrich (St. Louis, MO)
Lot/Batch #: 028K1411 (expiration date 8/4/2011)
Purity: 99.0%
CAS #: 57-63-6

4. Solvent/Vehicle Control (test substance): Methylcellulose

Supplier: Sigma Chemical (St. Louis, MO)
Lot/Batch #: 060M0123V (expiration date 5/1/2013)
Rationale (if other than water): Test substance not soluble in water at the concentrations used in the study
Final concentration: 0.5% (w/v)

Solvent/Vehicle Control (EE): Ethanol/Corn oil

Supplier: Spectrum Chemical Manufacturing Corporation (New Brunswick, NJ)
Lot #: Ethanol: ZT0426 (expiration date 8/2/2013)
Corn oil: 2AD0465 (expiration date 2/1/2013)
Rationale (if other than water): Not applicable
Final concentration: EE was dissolved in minimal amounts of 95% ethanol and then diluted with corn oil (ratio of ethanol to corn oil was not reported)

5. Test Animals:

Species:	Rat (ovariectomized females only)	
Strain:	Sprague Dawley [Crl:CD(SD)]	
Age/weight at dose initiation:	PND 66-67/ 245.6 – 301.2 g	
Source:	Charles River Laboratories (Portage, MI)	
Housing:	Rats were individually housed in stainless steel wire-mesh cages suspended above cage board.	
Diet:	2016C*M* Teklad Global 16% Protein Rodent Diet, Harlan Laboratories, *ad libitum* Genistein equivalent content = 29.0 ppm total isoflavones (genistein + daidzein + glycitein)	
Water:	Reverse-osmosis purified drinking water, *ad libitum*	
Environmental conditions:	**Temperature:**	21.3-21.6 ºC (mean daily temperature)
	Humidity:	51.8-55.2% (mean daily humidity)
	Air changes:	10/hr
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	11 days	

B. STUDY DESIGN

1. In-Life Dates: Start: July 2, 2011 End: July 5, 2011

2. Study Design: Sexually mature ovariectomized female rats were received from Charles River Laboratories; rats were ovariectomized at PND 49 by the supplier. Animals were received approximately one week following ovariectomy (PND 55-56) and acclimated for 11 days prior to initiation of dosing. Vaginal smears were taken daily for five days prior to assignment of animals to study, to verify that females were in persistent diestrus. The dose administration period was from PND 66-67 through 68-69. Rats were euthanized approximately 24 hours later on PND 69-70 and necropsied for uterine weight measurements

3. Animal Assignment: Animals were randomly assigned, stratified by body weight, to the test groups noted in Table 1. Statistical analysis indicated that there were no significant differences between group mean weights at study initiation. Furthermore, the body weight of each animal was within ±20% of the overall mean.

TABLE 1. Study Design[a]

Test Group	Dose (mg/kg/day)	# of Females
Estrogen Agonist Assay		
Vehicle Control	0	6
Low Glyphosate	100	6
Mid Glyphosate	300	6
High Glyphosate	1000	6
17α-ethynyl estradiol (EE), Reference Estrogen	0.003	6

a Data were obtained from page 25 of the study report. Glyphosate concentrations are expressed as free base equivalents.

4. **Dose Selection Rationale:** The dose levels used in this study were chosen based on the results of a dose range-finding study.[1] In the study, the test substance was administered by oral gavage to four groups of female rats [Crl:CD(SD)] at 0, 200, 500, and 1,000 mg/kg/day once daily for 3 consecutive days; the 0, 200, and 500 mg/kg/day dosing groups consisted of 5 rats each and the 1,000 mg/kg/day group consisted of 8 rats. All females survived to the scheduled necropsy. Mean body weights, body weight gains, and food consumption in all treatment groups were similar to the control group. Therefore, the high-dose level of 1,000 mg/kg/day (limit dose) was selected for the current study.

5. **Dose Preparation and Analysis:** Dose formulations were prepared once as single formulations for each dosage level by mixing appropriate amounts of test substance with 0.5% methylcellulose. A stock solution of EE was prepared once by dissolving the material in a small amount of 95% ethanol and diluting to volume with corn oil; dosing formulations were prepared daily by diluting the stock solution. Analyses to demonstrate homogeneity, stability, and resuspension homogeneity were conducted previously for dose formulations at 1 and 200 mg/mL following up to 15 days of room temperature storage.[2] During the study, samples of each test substance dosing formulation (middle stratum of each) prepared during the in-life phase were analyzed for achieved concentration.

Results of Dose Analysis

Homogeneity: Not provided

Stability: It was stated that glyphosate in 95% methylcellulose at 1 and 200 mg/mL was stable at room temperature for 15 days.

Concentration (% of nominal): 104-105%

The analytical data indicated that the variation between nominal and actual dosage to the animals was acceptable. The study referenced above should be submitted for verification of the homogeneity and stability findings.

6. **Dosage Administration:** Animals were administered the test formulations and/or EE or vehicle daily via oral gavage for three consecutive days in a dose volume of 5 mL/kg body weight. Dose volumes were adjusted daily based on the concurrent body weight measurement.

7. **Statistics:** Statistical analyses were conducted for organ weights, daily body weights, and body weight gains. Each endpoint was tested for homogeneity of variance using Levene's test. If that test was significant at p=0.01, then a log transformation was applied and

1 Stump, D.G. A Dose Range-Finding Oral (Gavage) Toxicity Study of Glyphosate in Young Adult Rats for the Endocrine Disruption Screening Program (Study No. WIL-843001). WIL Research Laboratories, LLC, Ashland, OH, 2011.

2 Haubenstricker, M.E. Analytical Validation and Stability Study of Glyphosate in Aqueous Formulations (Study No. WIL-843004). WIL Research Laboratories, LLC, Ashland, OH, 2011.

Levene's test conducted on the transformed data. If that test was still significant, then the square root transformation was applied to the raw data and Levene's test conducted again. If the test was still significant, then a nonparametric test, as described below, was used to analyze the data. One-sided tests were conducted for uterine weights and two-sided tests were conducted for body weights and body weight gains.

For uterine weights, if variances were homogeneous, the analysis of covariance (ANCOVA), using the body weight at termination as the covariate was performed; the two groups were compared using a one-sided Dunnett's test. For body weight and body weight gain data, if variances were homogeneous, an ANOVA was performed on data; the ANOVA test was followed by a two-sided Dunnett's test. If the transformations were unsuccessful in making the variances homogeneous, the nonparametric one- or two-sided Wilcoxon rank sum test was used to compare data for the positive control to the negative control. For comparison of dose groups to the negative control, if the transformations were unsuccessful in making the variances homogeneous, the nonparametric Kruskal-Wallis test was used, followed by a one- or two-sided Dunn's test. Significance was denoted at $p<0.05$. Statistical analyses were performed using SAS software (version 9.2 or higher). The statistical analyses were considered adequate.

C. METHODS

1. **Clinical Examinations:** Cage-side checks for mortality and moribundity were conducted twice daily. Individual clinical observations (hand-held physical examinations) were recorded daily through termination. Each rat was also observed for signs of toxicity approximately 4 hours following dosing.

2. **Body Weight:** Animals were weighed at randomization, daily throughout the dosing period, and at termination. Mean body weight changes were calculated for each corresponding interval and also for the overall treatment period (Days 0-3).

3. **Food Consumption (Optional):** Food consumption was not measured.

4. **Necropsy and Measurement of Uterine Weight:** On PND 69-70 (approximately 24 hours after final administration of the test substance), all surviving animals were euthanized by carbon dioxide inhalation and subjected to a gross necropsy. Dissection of the uterus was performed according to the U.S. EPA Guideline. Briefly, the vagina was removed just below the cervix in order to retain the luminal fluid in the uterus. The "wet" uterus (i.e., containing the luminal fluid) was weighed. Subsequently, the uterine horns were cut longitudinally and gently blotted with moist filter paper to remove the luminal fluid while preventing desiccation and the blotted uterus was weighed. The uterus and vagina were preserved in 10% neutral buffered formalin for possible future histopathologic examination.

5. **Microscopic Examination (Optional):** Microscopic examinations were not conducted.

II. RESULTS

A. OBSERVATIONS

1. Mortality: All animals survived until scheduled termination.

2. Clinical Signs of Toxicity: No test-substance related clinical signs of toxicity were observed in animals for any dose groups. Findings noted in the treated groups were limited to observation of red material around the nose in one rat in the 300 mg/kg/day dose group on one study day.

B. BODY WEIGHT AND WEIGHT GAIN: Body weight and body weight gain data are presented in Table 2. Body weights in the treatment groups were comparable to controls throughout the duration of the study. In the positive control (EE) group, mean body weights decreased (NS) on Days 3 and 4, leading to an overall body weight loss during the study of −5.6 g ($p<0.01$) compared to a gain of 11.3 g in the controls.

TABLE 2. Selected Group Body Weights and Cumulative Body Weight Gains (g) In the Estrogen Agonist Assay [a]

Study Day #	Dose (mg/kg/day)														
	Vehicle Control			Glyphosate (100)			Glyphosate (300)			Glyphosate (1000)			Reference Estrogen EE (0.003)		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
1	6	277.0	19.2	6	277.6	14.6	6	275.2	15.6	6	278.3	15.5	6	278.7	18.8
2	6	279.7	19.5	6	282.4	15.8	6	279.2	16.9	6	282.8	16.6	6	280.2	21.1
3	6	286.4	21.1	6	288.2	16.3	6	284.8	15.5	6	287.5	16.5	6	278.2	19.4
4	6	288.3	21.0	6	292.9	16.0	6	284.3	19.4	6	291.6	17.5	6	273.2	20.9
Body Weight Gain (1 - 3)	6	11.3	3.6	6	15.3	3.0	6	9.1	5.1	6	13.3	6.0	6	-5.6**	3.4

a Data were obtained from Tables S6-S9 on pages 49-54 of the study report.
N Number of animals in the group
SD Standard Deviation
** Significantly different from controls at $p<0.01$

C. FOOD CONSUMPTION (Optional): Food consumption was not measured.

D. PATHOLOGY

1. Uterine Weights: Uterine weight data are presented in Table 3. Uterine weights in the glyphosate treatment groups were comparable to the vehicle controls. Absolute wet and blotted uterus weights for the positive control (EE) group were increased ($p<0.01$) by 758% and 256%, respectively. The increased uterine weights were within the expected range.

No macroscopic findings in the uterus were observed in the glyphosate treatment groups or the positive control group.

TABLE 3. Uterine Weights from Estrogen Agonist Assay in Sprague Dawley Rats[a]

Parameter	Dose (mg/kg/day)														
	Vehicle Control			Glyphosate (100)			Glyphosate (300)			Glyphosate (1000)			Reference Estrogen EE (0.003)		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Terminal BW	6	289	21	6	293	16	6	284	19	6	292	18	6	273	21
Wet, absolute (mg)	6	111.0	10.8	6	110.7	12.5	6	118.3	16.5	6	113.6	9.7	6	953.1** (↑758)	90.4
Wet, relative (%)	6	0.038	0.0025	6	0.038	0.0056	6	0.042	0.0061	6	0.039	0.0044	6	0.352	0.055
Blotted, absolute (mg)	6	98.2	11.7	6	98.7	10.6	6	103.0	11.6	6	102.4	8.9	6	349.3** (↑256)	31.1
Blotted, relative (%)	6	0.034	0.0024	6	0.034	0.0048	6	0.0.36	0.0040	6	0.035	0.0038	6	0.129	0.017

a Data were obtained from Tables S11-S14 on pages 56-59 of the study report. Percent differences from controls, calculated by the reviewers, are included in parentheses.
BW Body weight
N Number of animals in the group
SD Standard Deviation
** Significantly different from controls at $p<0.01$

2. Microscopic Examination (Optional): Microscopic examinations were not conducted.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: Based on the lack of effects on mean uterine weights (wet and blotted), glyphosate did not demonstrate or mimic biological activities consistent with agonism of natural estrogens when administered orally to ovariectomized female rats at dosage levels of 100, 300, and 1,000 mg/kg/day. The positive control substance (17α-ethynylestradiol) elicited the expected increases in wet and blotted uterine weights (8.6- and 3.6-fold, respectively).

B. AGENCY COMMENTS: All animals survived until scheduled termination and no treatment-related clinical findings were observed in glyphosate dosed animals. Body weights, body weight gains, and uterine weights in the glyphosate dosing groups were comparable to the vehicle controls.

In the positive control (EE) group, mean body weights decreased (NS) on Days 3 and 4, leading to an overall body weight loss during the study of 5.6 g ($p<0.01$) compared to a gain of 11.3 g in the controls. Absolute wet and blotted uterus weights for the positive control (EE) group were increased ($p<0.01$) by 758% and 256%, respectively, as expected. No statistically significant changes were seen in uterine weight in this assay. Glyphosate is negative in the uterotrophic assay.

C. STUDY DEFICIENCIES: None